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Point Counter Point

What Can 18S rDNA Do for Bivalve Phylogeny?

In the July (1996) issue of *Journal of Molecular Evolution,* Steiner and Müller (1996) discuss the limitations of 18S rDNA sequences to achieve a ''reasonable'' phylogeny of bivalve mollusks. After a nice introduction to the subject and thorough references to molecular techniques applied to Bivalvia, they collect 21 18S rDNA sequences published to date—2 Polyplacophora, 3 Gastropoda, and 16 Bivalvia belonging to the Filibranchia (=Pteriomorphia) and Eulamelibranchia (=Heterodonta)—plus 2 new sequences (both also Filibranchia). Eight of these sequences, however, were excluded from the main analyses. Two more partial sequences were not included: one Cephalopoda and one Polyplacophora (Wheeler et al. 1993). Different tree-constructing algorithms, based on parsimony, neighbor-joining, and maximum-likelihood, as well as other methods, to test the reliability of the clades, such as the PRN and spectral analysis were used.

The results obtained led them to propose a ''preferred tree (their Fig. 9) combining the results from the 18S rDNA data and morphological characters compiled by Waller (1978, 1990).'' In their words, this tree ''is not a computer-constructed tree but an evaluation of hypotheses on bivalve phylogeny from the given morphological and molecular trees.'' Moreover ''the failure of neighbor-joining and parsimony to resolve this clade [Bivalvia]'' is probably due to the different substitution rates among species.

We have reanalyzed the data set presented by Steiner and Müller (1996), to which we added 12 more molluscan sequences available via GenBank. Sequence alignments were generated using ClustalW (Thompson et al. 1996), with subsequent manual correction based on secondary structure constraints. Two alignments were analyzed, one with the whole data set and another in which four ambiguous-alignment zones corresponding to loops were removed. Alignments are available at the ftp site porthos.bio.ub.es/users/ftp/pub/teu/18Sphylogeny. Parsimony analyses were performed with PAUP 3.1.1 (Swofford 1993). Analyses were run for both alignments, using unweighted and weighted parsimony at different transition/transversion rates (3 and 5), as well as using transversion parsimony (transitions downweighted to 0). Gaps were treated in two ways, as missing data or as a fifth state. Three independent data sets were analyzed: the 13 taxa analyzed in the original paper (data set 1), the 21 taxa reported in their Table 2 (data set 2), and all molluscan sequences published to date (data set 3).

All the analyses run with data sets 2 and 3 rendered Bivalvia monophyletic (Figs. 1B and C) with an internal unstable topology, varying depending on the analysis condition. The following clades above family level are always monophyletic: Pectinidae, *Arca* + Pectinidae, Ostreidae, Mytilidae, Mactridae, and the two represented Eulamelibranchia groups (*Mya* + Mactridae). The paraphyletic Bivalvia have only been recovered in some cases when data set 1 was used for the analyses (Fig. 1A). Although the internal topology differs slightly from their Fig. 4, the two Gastropoda also cluster with the two Mactridae, which are the longest branches of the tree. These results clearly indicate that the paraphyletic Bivalvia is an artifact, most likely due to the low number of taxa used in the analyses, combined with the long branch attraction effect among the Gastropoda and the Mactridae.

Simulations have demonstrated that adding large numbers of additional taxa to phylogenetic analyses may increase the accuracy of the estimated trees, reduce the long branch attraction effect, and, at the same time, reduce the need for computationally complex methods of analysis (Hillis 1996). In our case, solely increasing the number of taxa from 13 to 21 is enough to obtain a robust monophyletic Bivalvia clade. From these results we suggest that computational efforts in phylogenetic analysis should focus on analyzing the biggest available data sets instead of performing more costly computational methods (e.g., the PRN and spectral analyses) with fewer taxa.

The failure of recovering a reliable internal phylogeny

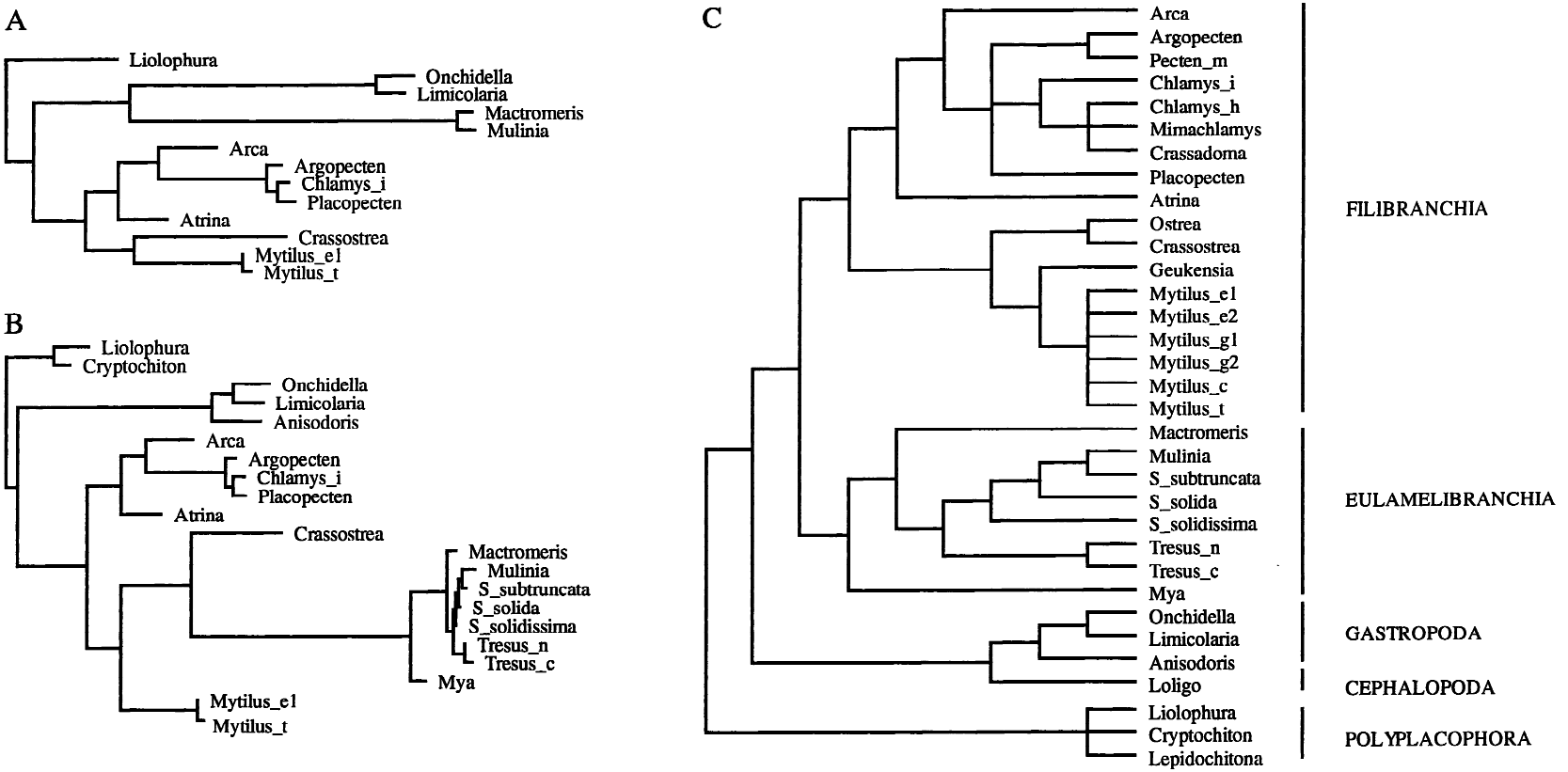

Fig. 1. **(A)** Single MPT (558 steps; CI = 0.719; RI = 0.753) obtained by branch-and-bound search using the complete alignment and unweighted parsimony, with the Bivalvia appearing as a paraphyletic group. **(B)** Single MPT (657 steps; CI = 0.686; RI = 0.0855) obtained by 100 replicates of stepwise addition in a heuristic search using the complete alignment and unweighted parsimony. Bivalvia is monophyletic, but with an unreliable internal topology. **(C)**Strict consensus tree of 18 MPTs (1605 steps) obtained by 10 replicates of stepwise addition in heuristic search using the complete alignment and weighted parsimony($T_S/T_V = 3$). Bivalvia is monophyletic, and the internal topology is better resolved with two clades, one representing the Filibranchia and another including the Eulamelibranchia. Accession codes to GeneBank, following the order in C, are X90960, L11265, L49053, L11232, L49049, L49051, L49050, X53899, X90961, L49052, X60315, L33450, L24489, L33448, L33451, L33452, L33449, L24490, L11230, L11268, L11271, L11266, L11270, L11269, L11267, M20094–M21541–M21175, X70211, X66374, M20097–M20098–M20099, *Loligo pealei* (Wheeler et al. 1993), X70210, M20056–20058, *Lepidochitona cavernae* (Wheeler et al. 1993).

of the bivalves by Steiner and Müller (1996) may be due to the sampling effort; only representatives of one (Lamelibranchia) of the three bivalvian subclasses (Protobranchia, Lamelibranchia, and Anomalodesmata) were included in the analyses. Another aspect to consider is the homology statements assigned in the alignment (which is not available at the ftp site described in the paper or via request, so it could not be examined).

The sampling deficiencies are also mentioned in the paper, so it is unclear why at least one sequence from the Protobranchia, the most primitive bivalves, was not included in their analysis. Also, the lack of sequences from Scaphopoda, the widely accepted sister group of the Bivalvia (i.e., Salvini-Plawen and Steiner 1996), may strongly affect the phylogenetic inference of the ingroup. In fact the Protobranchia, the most primitive group of bivalves, was used as outgroup for cladistic morphological analyses of the Scaphopoda (Steiner 1992, 1996). The choice of distant available outgroup sequences in many molecular studies has been pointed out previously by other authors. In many cases, a random outgroup sequence will join the longest branch of the ingroup; there may be so many changes along the branch connecting the ingroup to the outgroup that the sequences have become effectively randomized. In the worst case, this can lead to spurious long branch attraction effects with artificial rooting along longer ingroup branches (Wheeler 1990; Swofford et al. 1996). This seems to be the case for the clade (Gastropoda + Mactridae), which shows the longest branches in the cladogram, suggesting a long branch attraction effect. Thus, the choice of the outgroup taxa is fundamental in the analysis and must be chosen carefully, trying to minimize the impact of long branches.

Finally, we would like to mention that after such complex and time-consuming computational analyses (i.e., PRN and spectral analyses), Steiner and Müller proposed a ‘‘preferred tree’’ inferred by eye. It is well-known that there are scientific methods for combining independent sources of data for reconstructing phylogenetic scenarios. It can be done by a priori combination of data sets in a total evidence analysis or, alternatively, by a posteriori combining the independently generated trees using a consensus method (taxonomic congruence) to evaluate congruence areas among trees. A wide discussion of such approaches is given in the reviews by Eernise and Kluge (1993), Chippindale and Wiens (1994), de Queiroz et al. (1995), and Huelsenbeck et al. (1996), among others.

In conclusion, we strongly recommend the use of as many sequences as possible and we encourage accurately designed taxonomic sampling, both in the ingroup and in the outgroup, in order to perform the most accurate phylogenetic analyses.

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Gonzalo Giribet
Departament de Biologia Animal
Universitat de Barcelona
Av. Diagonal 645
08071 Barcelona, Spain

Salvador Carranza
Departament de Genètica
Universitat de Barcelona
Av. Diagonal 645
08071 Barcelona, Spain

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Response

Giribet and Carranza, in their response to Steiner and Mueller (1996), present an alternative alignment and evidence for bivalve monophyly inferred from 18S rDNA and express their doubts concerning the use of ‘‘. . . complex and time consuming computational analysis . . .’’ (Giribet and Carranza 1999, p. 256). I have to apologize